From Page No. 37

Checked mammalian fusion construct transformation plates

Started 40 x 5ml CB cult 50 (+ master) MP's

Inc all 37°C O/N.

Want to prepare HPTK6 full length in pSV15.T

need to prepare EcoR1/BamH1 vector

RD: mixed

10µl	vector DNA
10µl	10x H
75µl	H_2O
5µl	EcoR1
100	

Inc 37°C O/N.

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From Page No. 37

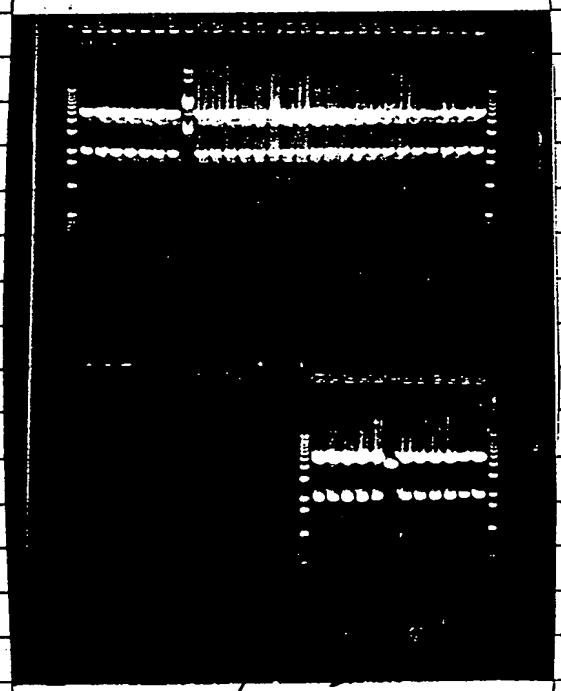
Ran 40 O/N MP cultures in Autogen Cmasters to

Recovered each in ~200 µl TE

RD's
Per rxn
4µl MP DNA
2µl 10x B
0.5µl EcoRI
0.5µl HindIII
1 µl RNase A
12µl H2O
20

Inc 37°C ~1.5hrs → added 4µl dye to each

Ran 15µl each on 0.7% agarose (1x TBE)



only 2 negatives

Started 2x 500ml 2YT carb-50

Maxi's on #1 + #2

Inc 37°C O/N

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From Page No. 39

Want to subclone full length HAT K6 to pSV5.I.D.C for stable transfection.

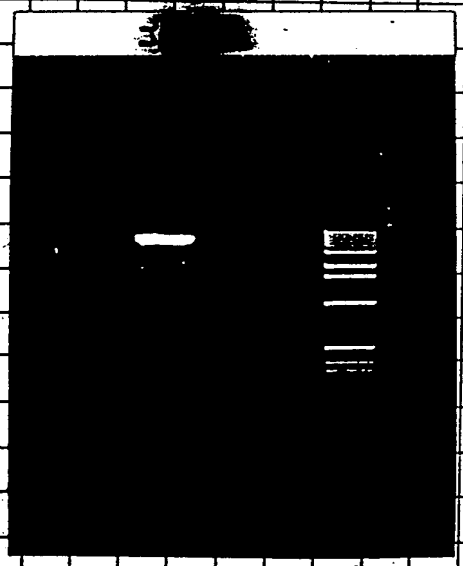
(Have RI/BamHI insert already prepared)

Vector digest:

mixed 4µl MP DNA (pSV5) (~1.5µg)
2µl 10x B
2µl BamHI
2µl EcoRI
12µl H2O
20

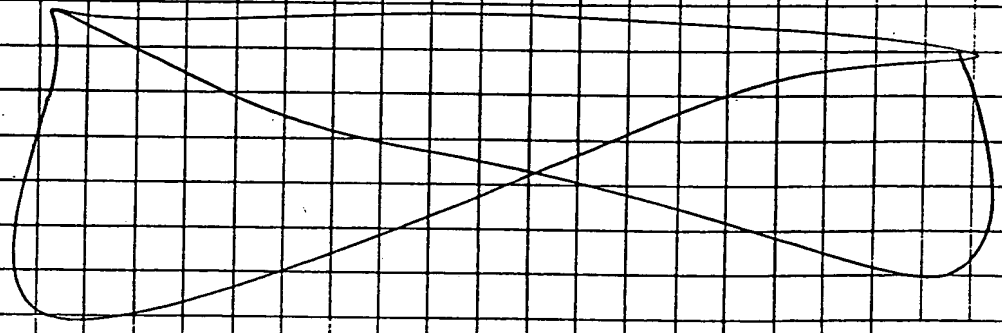
Inc 37°C 1.5 hrs → added 4µl dye

Ran entire rxn on 1% crp agarose (1x TBE)



cut out indicated band
D.A. Magic PCR prep
Rx'd in 50µl TE

Ligated to HAT K6 (Hi & Lo conc)
(plus vect. cont).
12.5°C O/N.



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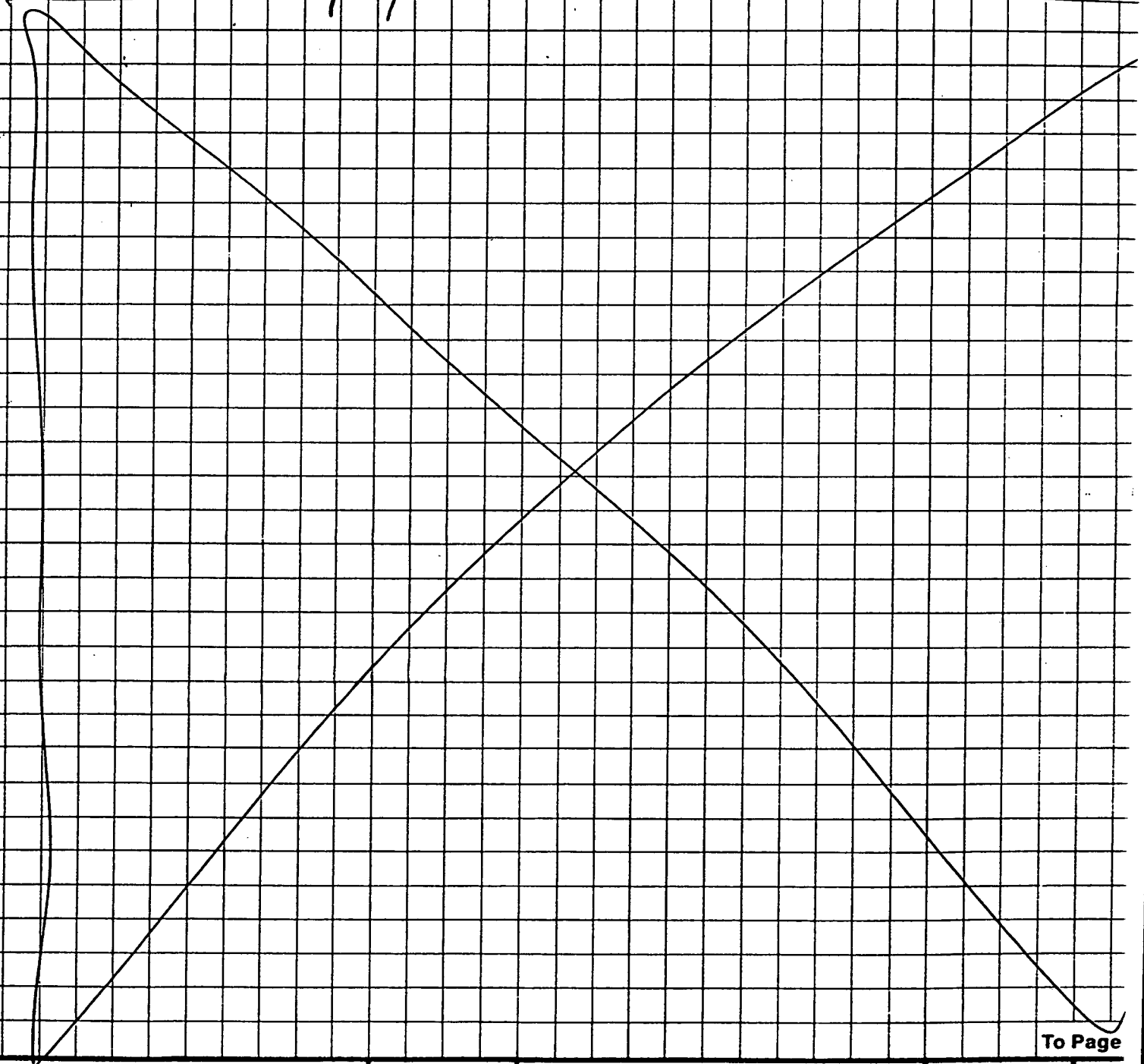
Recorded by [redacted]

From Page No. 40

Transferred O/N cells to -20°C.

Stored maxiprep cultures 4°C



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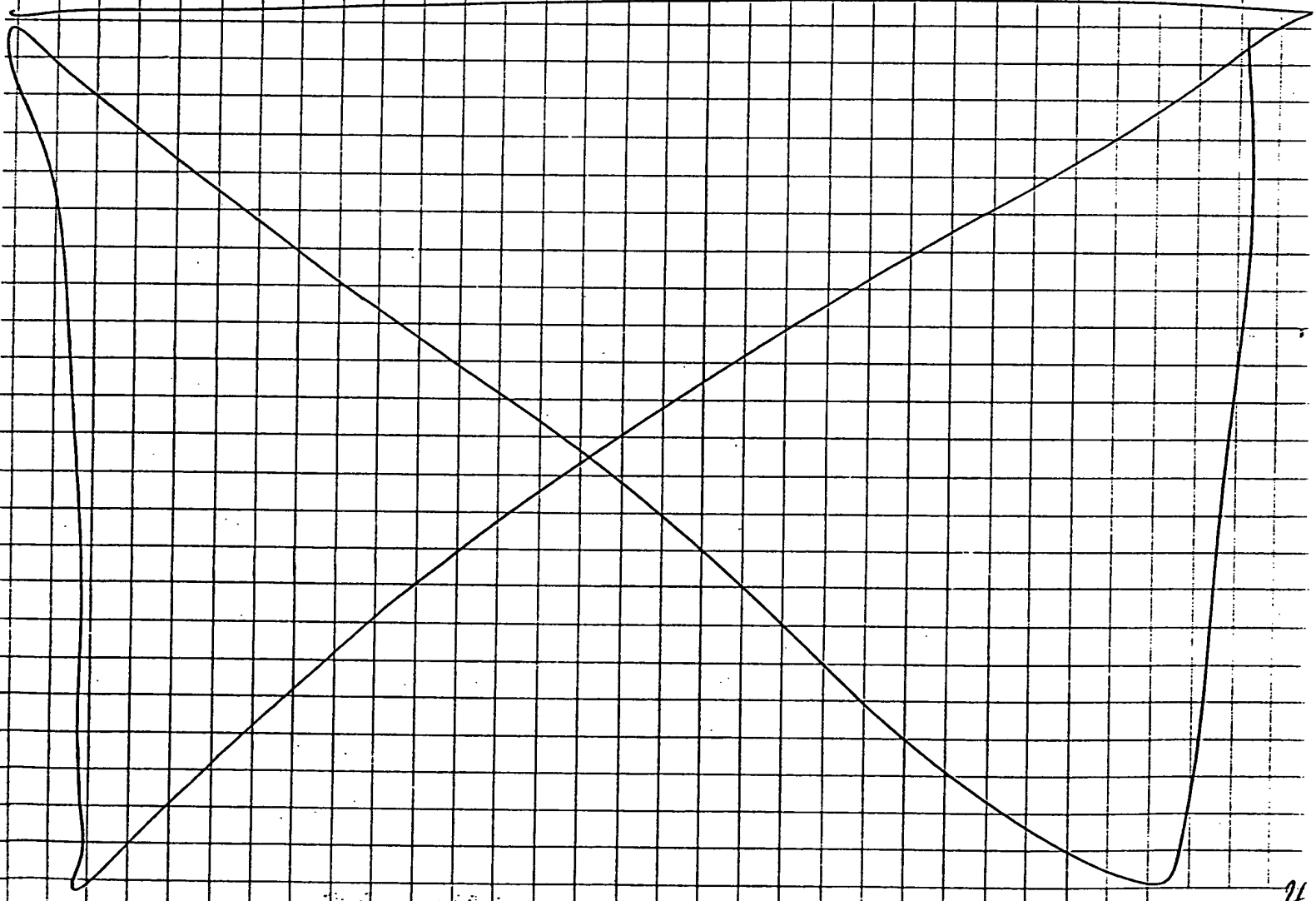
om Page No. 41

Transformed pSV15/HPTK6 (full length) into competent E. coli

Plated each onto 5 x 100mm LB carb 50 (control onto 1 plate)

Inc all (11) o/N 37°C.



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		Recorded by: [redacted]	

From Page No 44

Did magic MP's on 8 o/N pSV15 MP's (Master stored 4°C

RD's
2µl MP DNA
2µl 10x B
0.5µl BamHI
0.5µl EcoRI
15µl H_2O
20

Inc 37°C ~ 1.5hrs → added 4µl dye to each

Ran 15µl each on 0.7% agarose (1x TBE)

5 of 8 positive.

Started 1x 500ml LB carb 50 o/N M11

Inc 37°C o/N.

Received M. Mark's NorThern Blo
(Clontech mouse MTN)

Prehybridized according to
Clontech's protocol
42°C ~ 6 hrs.

Did Magic clean-up on o/N SacI
RD of pSEM-3Z HPTK6

Did SalI RD

Ran on 1% LMP agarose (1x TBE)

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